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(54) Title: METHODS FOR BREAST CANCER DETECTION

(57) Abstract

Methods are provided for detecting XMMBR-B55 antigen in serum. The presence of XMMBR-B55 in serum has been associated with breast and ovarian carcinoma. Conventional immunological detection techniques may be employed. XMMBR-B55 antibody is produced by XMBBR-B55 hybridoma cell line which is deposited at the American Type Culture Collection under assession number HB 9485.

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METHODS FOR BREAST CANCER DETECTION

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates generally to the diagnosis and monitoring of breast cancer. More particularly, the invention relates to the detection of a breast cancer tumor marker in serum based on identification of a unique epitopic site.

Breast cancer is one of the most common forms of cancer, afflicting approximately one of every 14 women in Western countries. Although a variety of effective treatments for breast cancer exist, early diagnosis is often essential in determining patient survival. Present diagnostic methods, including palpation and mammography, allow detection of tumor masses having a diameter on the order of 1 cm, but frequently even with such small tumors, metastasis may have already occurred. Thus, there is clearly a need for detection methods which allow for early identification of breast cancer. In particular, it would be desirable if the methods provided for a blood or serum assay which is relatively easy to perform and which can be used for routine screening of large populations of women.

Serum assays for cancer normally depend on detection of a substance, typically referred to as a tumor marker, which is characteristic of the cancer in some manner. The tumor marker is usually a protein, glycoprotein, polysaccharide, or the like, which is produced by cancer cells. Tumor markers may be variants of normal cell products, or may be the normal product which is produced in an abnormal manner. For example, production of the tumor marker substance may be greatly increased in the cancer cells in comparison to normal cells, or products normally confined within

the cell or cell membrane may be released into circulation.

A number of breast-associated tumor markers have been identified. Of particular interest to the present invention is a high apparent molecular weight (greater than 400 kd) antigen which has been associated with certain epithelial malignancies, particularly secretory glandular epithelial malignancies. The antigen (referred to hereinafter as XMMBR-B55 antigen) has been isolated from normal body fluids, including urine and skim milk in normal individuals free from cancer. In addition, the antigen is released into circulation from breast cancer cells. Several antibodies capable of recognizing the antigen have been prepared and used for detection in serum. These antibodies are discussed in more detail hereinbelow.

Immunologic detection of tumor markers in serum can frequently be problematic. Even with known tumor markers, detectability will vary depending upon the particular epitope which is bound by the antibody. Moreover, detection can often be further improved by employing a panel of antibodies capable of recognizing different epitopes on the same tumor marker antigen.

In view of the above, it would be desirable to provide assays capable of recognizing particular epitopes which provide enhanced detectability of the antigen. It would further be desirable to provide the serum assays employing a panel of antibodies, where at least some of the antibodies are capable of recognizing different epitopes on the same tumor marker antigen.

2. Description of the Background Art

The anti-breast carcinoma monoclonal antibody, XMMBR-B55, reacts with most malignancies of epithelial origin and also with the luminal surfaces of specialized epithelia. See, Ellis et al. (1984) Histopathol. 8:501-506 (Antibody XMMBR-B55 was previously designated as NCRC-11). The target antigen

for this antibody has been isolated from breast and ovarian carcinomas and it has been shown to be a high molecular weight glycoprotein. See, Price et al. (1985) Int. J. Cancer 36:567-574, and Price et al. (1986) Brit. J. Cancer 54:393-400. The antigen is immunologically complex and bears epitopes for a number of other monoclonal antibodies some of which were originally raised against products derived from human milk and others against human tumor cells. See, Price et al (1986) supra. Such cross-reacting monoclonal antibodies include HMFG-1 and HMFG-2 (Taylor-Papadimitriou et al. (1981) Int. J. Cancer 28:17; EMA/E29 (Heyderman et al. (1985) Br. J. Cancer 52:355); M8 (Edwards and Brooks (1984) J. Histochem. Cytochem. 32:531); 115D8 and 115F5 (Hilkens et al. (1984) Int. J. Cancer 34:197); Ca1 (Ashall et al. (1982) Lancet ii; 1); and Ca2, and Ca3 (Bramwell et al. (1985) Br. J. Cancer 48:177).

Antigens reactive with both HMFG-1 and HMFG-2 antibodies have been assayed in patients' sera. See, Burchell et al. (1984) Int. J. Cancer 34:763-768. Sandwich immunoassays using the 115D8 antibody have been employed to detect antigens in the sera of breast cancer patients. Hilkens et al., in: Proc. Intl. Workshop on Monoclonal Antibodies and Breast Cancer, Martinus Nijhoff, 1985, pp 28-42, and Hilkens et al. (1986) Cancer Res. 46:2582-2587. Elevated levels of 115D8 antigen were found with increasing frequency according to the staging of the disease so that up to 79% of sera from patients with advanced disease (Stage IV) were positive in the assay. Increasing or decreasing 115D8 antigen levels correlated with breast cancer progression or regression. Hilkens et al. (1986) supra.

SUMMARY OF THE INVENTION

Methods are provided for the early diagnosis and monitoring of neoplastic diseases, particularly

breast and ovarian carcinomas. The method relies on detection of a particular high molecular weight antigen (referred to hereinafter as XMMBR-B55 antigen) which is present in most normal epithelial cells and released into circulation by neoplastic epithelial cells, such as neoplastic breast and ovarian epithelia. Detection is based on the presence of an epitope or determinant on the antigen which is recognized by XMMBR-B55 monoclonal antibody (referred to hereinafter as XMMBR-B55 epitope). A wide variety of known immunological techniques may be employed for such detection, and the particular epitope may be detected in combination with one or more additional epitopes which are characteristic of the XMMBR-B55 antigen. Detection of XMMBR-B55 antigen through the XMMBR-B55 epitope appears to provide greater selectivity and sensitivity than that which is available through previously recognized epitopes.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a chart comparing the result of two-site immunoradiometric assays for XMMBR-B55 antigen in the sera from both patients and controls. Serum samples were tested at a dilution of 1/10 in panels A and C, and at a dilution of 1/5 in panel B.

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

A serum assay is provided for the detection of XMMBR-B55 antigen, which assay is useful for the early diagnosis and monitoring of cancer of the epithelial cells (carcinomas), particularly breast and ovarian carcinomas. XMMBR-B55 antigen is detected immunologically through recognition of a particular epitopic or determinant site which is recognized and defined by XMMBR-B55 monoclonal antibody. The assays of the present invention may rely on detection of the XMMBR-B55 epitope alone, or may rely on detection of two or more different epitopes characteristic of the XMMBR-B55 antigen, where at least one of the epitopes

is the XMMBR-B55 epitope. By relying on the recognition of two or more distinct epitopes, the selectivity and sensitivity of the serum assay may be further enhanced. In particular, it has been found that reliance on the XMMBR-B55 epitope will reduce the incidence of false positive results in individuals free from epithelial carcinomas.

XMMBR-B55 antibody is produced by the XMMBR-B55 hybridoma cell line which was deposited for patent purposes on July 23, 1987 at the American Culture Collection, Rockville, Maryland 20852. The deposit was granted accession number HB 9485.

The XMMBR-B55 antigen is a high molecular weight (greater than 400 kD) mucin (mucopolysaccharide) which is present in soluble form in the milk and urine of normal, healthy individuals, as well as in solubilized breast and ovarian subcellular membranes from neoplastic cells. The antigen is reactive with several monoclonal antibodies which have been previously described. These antibodies, together with the references in which they are described, are set forth in Table 1 hereinbelow.

TABLE 1

Antibodies which recognize distinct epitopes on the XMMBR-B55 antigen.

Antibody	Reference
115D8[1]	Hilkens et al. (1984) supra.
115F5[1]	"
HMFG1[1]	Taylor-Papadimitiou et al.
HMFG2[1]	(1981), supra.
EMA/E29[1]	Heyderman et al. (1985) supra.
M8[1]	Edwards and Brooks (1984), supra.
Ca1[2]	Ashall et al. (1982), supra.
Ca2[3]	Bramwell et al. (1985) supra.
Ca3[3]	"

[1] Raised against human milk fat globule

[2] Raised against glycoproteins from cultured laryngeal carcinoma (H.Ep2 cells).

[3] Raised against purified Ca1 defined antigen.

As demonstrated in the Experimental section hereinafter, XMMBR-B55 antibody binds to a distinct epitopic site on the XMMBR-B55 antigen which is not recognized by any of the antibodies in Table 1. Serum assays based on recognition of the XMMBR-B55 epitopic site appear to offer greater specificity for breast and ovarian tumor antigen than do assays based on any of the other antibodies capable of recognizing XMMBR-B55 antigen. It is believed that such specificity may derive from the fact that the XMMBR-B55 antibody was raised against breast tumor antigen, in contrast to the other antibodies which were raised against human milk fat globule or a laryngeal carcinoma cell line.

XMMBR-B55 antigen may be detected in patient blood, plasma or sera, usually sera, by conventional immunological techniques. A blood sample will be taken by conventional phlebotomy, and will usually be treated prior to testing. Typically, the blood sample will be

clotted to remove the cellular components and clotting factors, to produce a serum sample.

A wide variety of immunological techniques will be available for detection of the XMMBR-B55 antigen in sera, including various competitive and non-competitive protein binding assays which have been described in the scientific and patent literature. Such techniques include competitive radioimmunoassay (RIA), two-site immunoradiometric assays (IRMA), and enzyme-linked immunoabsorbent assays (ELISA). Specific immunological assay techniques are taught in U.S. Patent Nos. 3,791,932; 3,817,837; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,047; 4,089,876; 4,618,485; 4,650,751; 4,652,520; and 4,654,300.

An exemplary two-site immunoradiometric assay is set forth in the Experimental section hereinafter. In performing such two-site IRMA's, the XMMBR-B55 antibodies may be employed either as the capture or labelled antibody, or both. Oftentimes, it will be desirable to provide one or more distinct antibodies having specificity for other epitopes on the XMMBR-B55 antigen as either the capture antibody, labelled antibody, or both, in combination with the XMMBR-B55 antibody.

XMMBR-B55 antibodies suitable for the method of the present invention will usually be monoclonal antibodies specific for the XMMBR-B55 epitope, although highly purified monospecific polyclonal antibody specific for the epitope may also find use. Conveniently, XMMBR-B55 antibody obtained from the deposited XMMBR-B55 hybridoma cell line may be used, although other hybridoma cell lines producing monoclonal antibodies specific for the epitope may also be prepared. By specific for the epitope, it is meant that the antibodies will bind to the epitope with an affinity of at least about 10^7 liters/mole, more

usually at least about 10^8 liters/mole, and frequently 10^9 liters/mole and above.

In preparing hybridoma cell lines producing monoclonal antibodies specific for the XMMBR-B55 epitope, the XMMBR-B55 antibody from the deposited hybridoma cell line may be used to isolate antigen from a wide variety of natural sources, including neoplastic mammary and ovarian cells, normal human milk fat globules, antigen isolated from urine, and the like. Preferably, neoplastic cells are used as the source of the antigen as antibodies raised against a neoplastic source appear to provide enhanced selectivity for tumor marker. The antigen may then be used whole, or the epitope further purified by proteolytic degradation followed by affinity chromatographic purification of the epitope.

Once suitable antigen for use as an immunogen has been obtained, the antigen can be used to produce a hybridoma cell line producing antibodies specific for the XMMBR-B55 epitope by conventional techniques. The antigens may be injected into a wide variety of vertebrates, including mice, rats, sheep, and goats, and in particular mice. Usually, the animals are bled periodically with successive bleeds having improved titer and specificity. The antigens may be injected intramuscularly, intraperitoneally, subcutaneously, or the like. Usually, a vehicle is employed, such as complete or incomplete Freund's adjuvant.

Monoclonal antibodies are obtained by immortalizing spleen cells obtained from the immunized vertebrate. The manner of immortalization is not critical. Presently, the most common method is fusion with a myeloma fusion partner. Other techniques include EBV transformation, transformation with bare DNA, e.g., oncogenes, retroviruses, etc., or any other method which provides for stable maintenance of the spleen cell line and production of monoclonal

antibodies. The detailed technique for producing mouse x mouse monoclonal antibodies is taught by Oi and Herzenberg, in: "Selected Methods In Cellular Immunology," Mishell and Shiigi (eds.), W.H. Freeman and Company, San Francisco (1980, pp. 351-372. Antibodies useful in the present invention may be of any immunoglobulin class, i.e., IgG, including IgG1, IgG2a, and IgG2b, IgA, IgD, IgE, and IgM, usually being IgG or IgM.

EXPERIMENTAL

Materials and Methods

Monoclonal Antibodies

XMMBR-B55 (IgM) was originally prepared using spleen cells from a Balb/c mouse immunized against dissociated breast carcinoma cells (Ellis et al. (1984) supra.). The following anti-human milk fat globule membrane antibodies were employed: HMFG-1 (IgG1) and HMFG-2 (IgG1) (Taylor-Papadimitriou et al. (1981) supra.); an anti-human epithelial membrane antigen monoclonal antibody termed EMA (IgG2A) also known as E29 (Heyderman et al. (1985) supra.) available from Dakopatts (High Wycombe, Bucks.); LICR-LON-M8 (IgG1), LICR-LON-M18 (IgM) and LICR-LON-M24 (IgM) abbreviated to M8, M18 and M24 (Edwards and Brooks (1984) supra.) and 115D8 (IgG1), 115F5 (IgG2) and 115G2 (IgG2) (Hilkens et al. (1984) supra.) The antibody Ca1 (IgM) was prepared against wheat germ agglutinin binding glycoproteins from cultured human laryngeal carcinoma H.Ep2 cells (Ashall et al. (1982) supra.) and Ca2 (IgG1) and Ca3 (IgG1) were both prepared by immunization with the purified Ca1-defined antigen (Bramwell et al. (1985) supra.)

XMMBR-B55 antibody was purified from ascitic fluid by its binding to and elution from a Sepharose-lentil lectin affinity column (Pharmacia, Uppsala, Sweden), its protein concentration being determined assuming $E^{1\%}_{280nm}=11.9$.

Antigen Preparations

Fresh human milk was divided into cream and skim milk by centrifugation for 30 min at 1200g (Ashorn and Krohn (1985) Int. J. Cancer 35:179-184). The skim milk fraction was centrifuged at 100,000g for 60 min and stored at -20°C. The cream fraction was washed 5 times with phosphate buffered saline, pH 7.3 (PBS) (30 min at 1200g each time) to remove whey proteins and then quickly frozen. The cream was slowly thawed, rupturing membrane globules and vigorously shaken in PBS containing 1 mM $MgCl_2$. When butter formation was observed, the suspension was centrifuged at 100,000g for 60 min and the membranes were resuspended in PBS and stored at -20°C (Papsidero et al. (1983) Cancer Res. 43:1741-1747).

Normal urine was dialysed against PBS + 0.02% NaN_3 and centrifuged at 100,000g for 60 min before storage at -20°C.

Concentrations of protein solutions were determined from their absorbance at 280nm (assuming $E^{1\%}_{280nm}$=6.7, as for bovine serum albumin, (BSA) and protein concentrations for milk fat globule membranes were determined by the method of Lowry et al. (1951) J. Biol. Chem. 193:265-275, using BSA as standard.

XMMBR-B55 defined antigen preparations were isolated from detergent (Nonidet P-40) solubilized subcellular membranes from breast and ovarian mucinous carcinomas by immunoadsorbent chromatography using Sepharose-linked XMMBR-B55 antibodies as previously described in Price et al. (1986), supra. Samples of skim milk and normal urine were also employed as the starting material for XMMBR-B55 antigen isolation although detergent was not included in the initial sample solution or washing buffers. In all cases, XMMBR-B55 antigens were dialysed overnight against PBS, centrifuged at 100,000g for 60 min and stored at -20°C (Price et al. (1986) supra.

Radioisotopic Antiglobulin Assay

Milk fat globule membranes, skim milk (at 50 μg/ml) were adsorbed to Terasaki microtest plates (Price et al. (1986) supra.). Briefly, the radioisotopic antiglobulin assay was performed as follows: after antigen adsorption, the wells were washed 4 times with a washing buffer of PBS + 0.1% rabbit serum (RbS) + 0.02% NaN_3. During the final wash cycle, the wells were incubated for 30 min with washing buffer to complete the blocking of non-specific adsorption binding sites.

Monoclonal antibodies or washing buffer were added at 10 μl/well. All monoclonal antibodies were added at concentrations or dilutions predetermined to be at saturation (i.e., neat hybridoma supernatants, ascitic fluids at 1/1000 and purified IgG or IgM antibodies at 1 or 5 μg/ml respectively). After incubation for 1 to 2 hr at room temperature, the wells were aspirated and washed 4 times with washing buffer. ^{125}I-labelled affinity purified $F(ab')_2$ fragments of rabbit anti-mouse Ig were added at 10^5 c.p.m./10 μl/well (radioiodination of this reagent was performed using the chloramine T procedure of Jensenius and Williams (1974) Eur. J. Immunol. 4:91-97, using 18 MBq ^{125}I per 25 μg protein). Incubation was continued for 1 to 2 hr at room temperature. The wells were then aspirated, washed 6 times, after which the radioactivity in each well was determined. The non-specific binding of antibodies to "PBS-coated" and "BSA/RbS-blocked" wells was determined and the values obtained were subtracted from those determined with antigen-coated, BSA/RbS-blocked and antibody-treated wells.

Double Determinant or "Sandwich" Radioimmunoassays

XMMBR-B55 ascitic fluid (1/1000 in PBS + 0.02% NaN_3) was adsorbed onto the wells of Terasaki microtest plates. After incubation at 5°C for 18 hr,

the wells were aspirated and washed 4 times with washing buffer. On the 4th wash cycle, the plates were incubated with the washing buffer for 1 hr in order to block any remaining non-specific binding sites. Aliquots (10 µl) or normal or breast cancer patients' sera (at a dilution of 1/5 or 1/10) or washing buffer alone were added to the wells. Patients' sera was obtained from advanced breast cancer patients with progressive disease at the time of sampling. After incubation for 1 hr at room temperature, the wells were aspirated and washed 4 times. ^{125}I-XMMBR-B55 antibody (radiolabelled according to Fraker and Speck (1978) Biochem. Biophys. Res. Comm. 80:849-857), was added at 10^5 c.p.m./10 µl/well and incubated for 1 hr at room temperature. The wells were then aspirated, washed 6 times after which the radioactivity in each well was determined.

Immunoblotting

Urine samples (concentrated approximately 15-fold), skim milk (at 2 mg/ml) or XMMBR-B55 antigen preparations (in the range 10 to 100 µg/ml) were diluted 1:1 in SDS PAGE reducing sample buffer and then applied to 5 to 15% gradient polyacrylamide gels, with 3% stacking gels (which had been pre-run for 15 hr at 50 volt). Electrophoresis was continued at 50 volt for a further 15 hr at 50 volt using the discontinuous buffer system of Laemmli (1970) Nature 227:680-685).

Electroblotting onto nitrocellulose membranes was performed essentially as described by Towbin et al. (1979) Proc. Natl. Acad. Sci. USA 76:4350-4354 using the Bio-Rad Transblot Apparatus for 20 hr at 50 volt and 200 mA in 22 mM Tris, 192 mM glycine buffer, pH 8.3, containing 20% methanol. The membranes were blocked for 1 hr at room temperature using 1% BSA in PBS, and then washed 6 times over 30 min. Incubation with XMMBR-B55 hybridoma tissue culture supernatant was carried out for 3 hr at room temperature and thereafter

the membranes were washed as before. Horseradish peroxidase conjugated to $F(ab')_2$ fragments of sheep anti-mouse Ig (Amersham International, Amersham, Bucks., UK) at a dilution of 1/1000 in 1% BSA in PBS was applied to the nitrocellulose membranes and incubated for 1 hr. After washing 9 times with PBS over 45 min, peroxidase activity was detected by addition of 25 mg of 3,3'diaminobenzidine hydrochloride (B.D.H., Poole, Dorset, UK), in 50 ml 10 mM Tris-HCl, pH 7.4 with 50 μl 30% H_2O_2.

RESULTS

XMMBR-B55 antigens were isolated and purified from breast and ovarian carcinomas. These showed a characteristic profile of reactivity with a variety of monoclonal antibodies against products derived from human milk and the antibodies Ca1, Ca2 and Ca3 originally prepared against glycoproteins human laryngeal carcinoma cells (Ashall et al. (1982) supra.). The findings, presented in Table 2, are in accord with previous results using the same panel of antibodies (Price et al. (1986) supra.). This panel of antibodies was also tested against milk fat globule membranes, skim milk and normal urine. All 13 of the antibodies examined reacted with epitopes in products from human milk, with antibodies HMFG-1 and 115D8 being the most reactive. The antibodies M18 and M24 which were of minimal reactivity with the purified antigens, bound strongly to milk fat globule membranes and skim milk (Table 2). The reactivity profile of the panel of antibodies with normal urine was similar to that obtained with the antigens isolated from breast or ovarian tumors.

TABLE 2

Reactivity of a panel of monoclonal antibodies with various antigen preparations

Monoclonal Antibody	Mean cpm ± SD (-background) bound to: Breast Carcinoma*	Ovarian Carcinoma*	Milk fat Globule Membranes*	Skim Milk*	Normal Urine*
XMMBR-B55	7705±96	7274±116	3252±275	1428±54	2206±211
HMFG-1	6207±464	9380±257	13182±2099	11393±814	1213±147
HMFG-2	7551±107	7485±449	6712±213	4811±359	3087±328
EMA	8954±166	7786±214	10772±1158	5615±341	2588±230
115D8	15330±471	13238±305	13674±617	12627±495	3697±206
115F5	6963±299	6343±467	14003±453	7780±441	1807±264
115G2	598±150	2843±105	6407±559	7622±286	192±78
M8	7089±48	7176±241	9991±294	4221±198	2807±247
M18	291±31	1980±235	6884±224	7309±349	572±58
M24	646±161	117±61	5577±161	6142±378	277±102
Ca1	6107±40	3867±232	5799±625	2343±81	415±56
Ca2	6818±219	6954±206	2882±297	1037±126	2984±312
Ca3	7072±246	5946±38	3760±146	2199±73	2120±259

* Source of XMMBR-B55 antigen.

The nature of the XMMBR-B55 defined antigens in urine samples was examined by SDS PAGE, followed by electrophoretic transfer onto nitrocellulose membranes which were "stained" using the XMMBR-B55 antibody. The XMMBR-B55 antigens were identified in normal urine samples either as a single strong band of high apparent molecular weight (greater than 400kD) or as a doublet (with an indication of minor high molecular weight bands) and the mobility of these varied slightly between individuals. These variations and banding patterns of components reacting with the XMMBR-B55 antibody, resemble the genetic polymorphism in the expression of peanut lectin-binding urinary mucins (i.e., products of the "PUM" locus (Karllson et al. (1983) Ann. Hum. Genet. 47:263-269), and recent studies have established that this system may be defined using the peanut lectin and tumor-binding monoclonal antibodies (Swallow et al. (1986) Disease Markers 4:247-254) including XMMBR-B55.

XMMBR-B55 defined antigens were purified from both skim milk and normal urine by their binding to and elution from an immunoadsorbent of Sepharose linked XMMBR-B55 antibodies. The purified antigens (which were isolated from specimens obtained from single individuals, rather than from pooled samples) were examined by SDS PAGE and immunoblotting using the XMMBR-B55 antibody. The various antigens tested, including ovarian mucinous carcinoma antigen, skim milk antigen, and normal urine antigen, exhibited either one or two major bands of high apparent molecular weight (greather than 400kD) reactive with the XMMBR-B55 antibody. These banding patterns were reproducible on repeated testing and there was no evidence for a trivial explanation (e.g., sample deterioration) for the presence of doublets in purified antigens or unfractionated samples.

The purified antigens from skim milk and urine were examined for their reactivity with the panel of antibodies which had been employed to probe epitopes on purified antigens from breast and ovarian carcinomas. As shown in Table 3, the profile of reactivity of antibodies with these antigens from healthy individuals was similar to that obtained using antigens from tumors (Table 2). Notably the antibody 115D8 consistently displayed high binding with all antigens tested. XMMBR-B55 showed comparatively lower binding with purified skim milk antigens in relation to its reaction with the other antigens (Tables 2 and 3). The antibody M18, which reacts with Galβ1-4GlcNAcβ1-6- sequences (Foster and Neville (1984) Hum. Pathol. 15:5022-513) was reactive with all XMMBR-B55 defined antigens with the exception of that from breast carcinoma (Tables 2 and 3) although previous studies have shown that this sequence is masked by sialic acid in breast carcinoma antigens and may be exposed by neuraminidase treatment (Price et al. (1986) supra.). The determinant defined by the M24 antibody was apparently only found on the purified antigen isolated from skim milk and was not present on any of the other antigen preparations.

TABLE 3

Reactivity of a panel of monoclonal antibodies with XMMBR-B55 defined antigen preparations isolated from skim milk and normal urine

Monoclonal Antibody	Mean cpm ± SD (-background) bound to XMMBR-B55 defined antigen preparations isolated from: Skim Milk	Normal Urine
XMMBR-B55	2514±372	9715±488
HMFG-1	6519±208	12158±295
HMFG-2	3701±60	9523±542
EMA	4362±81	10823±126
115D8	9363±365	15145±391
115F5	3798±206	9492±141
115G2	5370±230	2730±278
M8	4934±304	10125±638
M18	5107±249	2395±64
M24	4235±200	243±159
Ca1	1098±152	3951±134
Ca2	2534±177	15871±339
Ca3	2844±217	9589±229

Clearly, XMMBR-B55 defined antigens obtained from detergent solubilized tumor membrane preparations were similar to XMMBR-B55 defined antigens isolated directly from skim milk or normal urine, neither of which had been treated with detergent to render the antigens soluble. Therefore, the antigen may be secreted or shed in a soluble form from tissues.

SDS PAGE analysis and Western blotting of serum samples from normal individuals and advanced breast cancer patients indicated that patients' serum contained detectable high molecular weight components

reactive with the XMMBR-B55 antibody whereas similar components in normal sera were not identified or only barely demonstrable, with at best, only extremely faint staining of the nitrocellulose membranes.

Serum samples were also analysed using a "sandwich" radioimmunoassay with XMMBR-B55 antibodies bound to the wells of microtest plates; these were treated with diluted serum samples and the capture of XMMBR-B55 antigens from the sample was then detected by the binding of ^{125}I-labelled XMMBR-B55 antibodies. Figure 1 summarizes the results of three such tests. Firstly, in panel a, serum samples were tested at a dilution of 1/10 and in panel b, samples were tested at a dilution of 1/5. With both approaches, the specificity of the assays was excellent with only one of the normal serum samples above the upper limit of normal reactivity, this being defined as the mean value for normal control samples plus two standard deviations. The sensitivity of these assays was however such that 41% of samples in panel a and 51% of samples in panel b displayed values above the upper limit establishing using the normal serum samples. The data in panel c describe the results of two tests separated in time by six months and performed with different batches of reagents. The patient samples gave similar results in the two tests and the control groups (laboratory controls in the first test and age matched controls in the second test - panel c), although small, also generated comparable ranges for normal values.

The assay described exhibits a low false positive rate of tumor detection in the population rate of tumor detection in the population of normal individuals. (Fig. 1). Also, XMMBR-B55 epitopes are under-represented in antigens derived from normal breast epithelia (e.g., skimmed milk, milk fat globule membrane) in comparison to those epitopes defined by

antibodies originally prepared against HMFG (e.g., 115D8, HMFG-1; Tables 2 and 3), so increased specificity for tumors is indicated in assays exploiting XMMBR-B55 epitope.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A method for determining neoplastic disease status in a human host, said method comprising detecting the presence of XMMBR-B55 epitope in a blood specimen from the human host.

2. A method as in claim 1, wherein the blood specimen is a serum sample.

3. A method as in claim 1, wherein the neoplastic disease is breast or ovarian carcinoma.

4. A method as in claim 1, wherein the presence of XMMBR-B55 epitope is detected by reaction with antibody specific for XMMBR-B55 epitope.

5. A method for detecting and monitoring breast and ovarian carcinomas in a human host, said method comprising:

exposing a blood specimen from the host to an antibody specific for the XMMBR-B55 epitope; and

detecting reaction between XMMBR-B55 antigen in the blood specimen and the antibody.

6. A method as in claim 5, wherein the blood specimen is a serum sample.

7. A method as in claim 5, wherein the antibody is XMMBR-B55 monoclonal antibody.

8. A method as in claim 5, wherein the antibody is exposed to the blood specimen in a non-competitive binding immunoassay.

9. A method as in claim 5, wherein the antibody is exposed to the blood specimen in a competitive binding immunoassay.

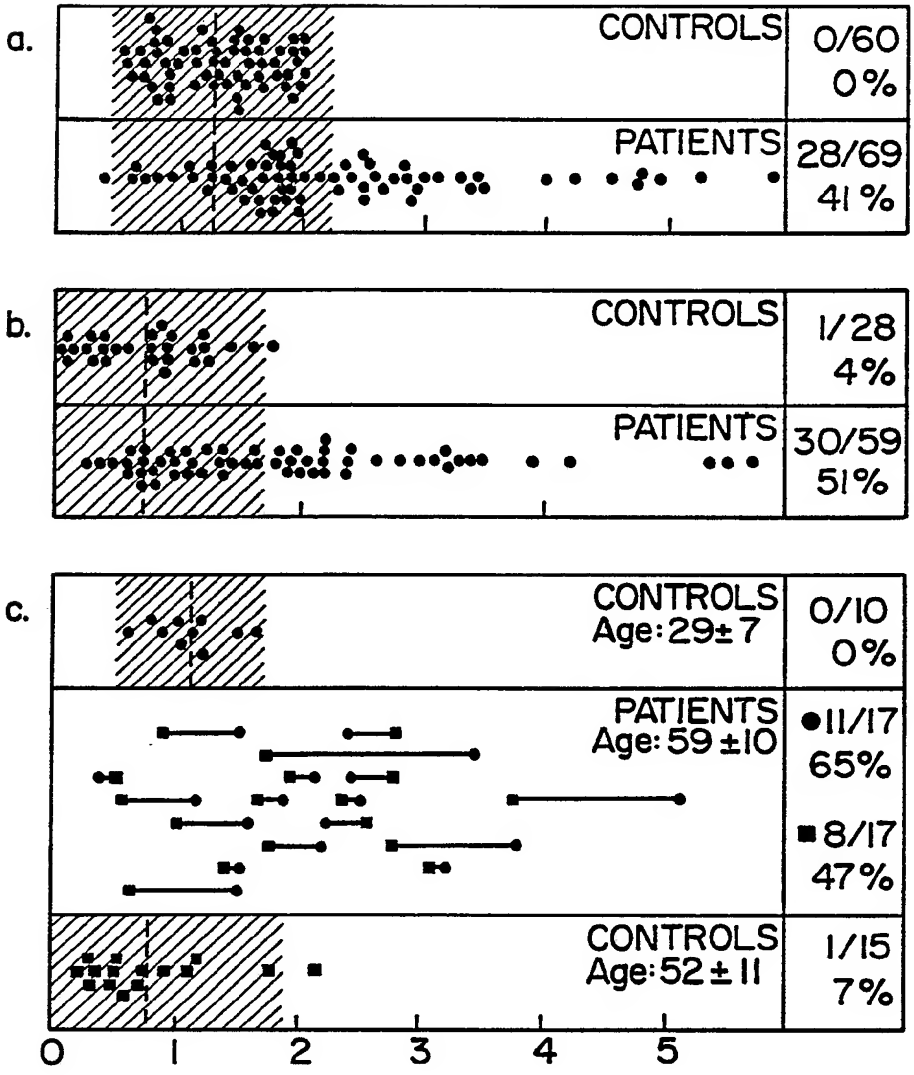
a.
CONTROLS
0/60
0%
PATIENTS
28/69
41%
b.
CONTROLS
1/28
4%
PATIENTS
30/59
51%
c.
CONTROLS
Age: 29±7
0/10
0%
PATIENTS
Age: 59±10
●11/17
65%
■8/17
47%
CONTROLS
Age: 52±11
1/15
7%
0
1
2
3
4
5
RADIOACTIVITY BOUND (cpm, x 10⁻³)

FIG. 1.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US88/02488

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(4): G01N 33/53 G01N 33/543 G01N 33/577 C07K 15/14
U.S. CL.: 435/7 436/518, 548 530/387

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	435/7, 68; 436/518, 519, 548, 813; 530/387; 935/110

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X Y	Cancer Research, Vol. 46, May 1986, HILKENS ET AL., "MAM-6 Antigen, A New Serum Marker For Breast Cancer Monitoring", pages 2582-2587, See page 2582, Column 1, lines 1-3, 23-25, 47-50, column 2, lines 15-20, and page 2584, lines 28-32.	1-9 1-9
Y	BR. J. CANCER., Vol. 54, 1986, (THE MACMILLAN PRESS LTD.), PRICE ET AL., "Epitope Analysis of Monoclonal Antibody NCRC-11 Defined Antigen Isolated from Human Ovarian and Breast Carcinomas", pages 393-400, See page 393, column 1, lines 10-15, column 2, lines 5-9, lines 17-23, page 395, Table 1, and page 399, column 2, lines 6-8 and lines 14-22.	1-9

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
8 September 1988	09 NOV 1988
International Searching Authority	**Signature of Authorized Officer**
ISA/US	Florina B. Hoffer FLORINA B. HOFFER

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	INT. J. CANCER, Vol. 36, 1985, PRICE ET AL., "Multiple Epitopes On A Human Breast-Carcinoma-Associated Antigen", pages 567-574, See page 569, column 2, lines 9-11, and page 572, column 2, lines 6-14.	1-9
Y	INT. J. CANCER, Vol. 34, 1984, DURCHELL ET AL., "Detection of the Tumour-Associated Antigens Recognized by the Monoclonal Antibodies HMFG-1 and 2 In Serum From Patients with Breast Cancer", pages 763-768, see page 763, column 1, lines 1 and 2, column 2, lines 32-40, and page 765, Table 1.	1-9
Y,P	US, A, 4,707,438 (KEYDAR) 17 November 1987 (column 4, lines 45-59, column 5, lines 7-28).	1-9
Y	US, A, 4,657,851 (FELLER ET AL.) 14 April 1987 (column 2, lines 14-26 and 48-54, column 5, lines 24-32).	1-9
A	US, A, 4,584,268 (CERIANI ET AL.) 22 April 1986 (column 1, lines 56-68, column 2, lines, 23-27, column 3, lines 27-43).	1-9